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Research Note

Recrystallization of amylopectin in concentrated starch gels

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The relation between the recrystallization of amylopectin and the increase in stiffness of starch gels during storage was studied by various techniques. From transmission electron microscopy it was concluded that the size of the crystalline domains in retrograded 30% w/w potato starch gels was about 5 nm, much smaller than those present in native starch. The super-helical structure formed by the crystalline domains in native starch granules was not seen. It may thus be concluded that in retrograded starch gels the long range ordering is not regained during retrogradation. The relation between the degree of recrystallization, as determined with differential scanning calorimetry (DSC), and the increase in stiffness, as measured in compression, was found to be closely related. Two mechanisms will be discussed which may explain these observations. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Nearly all green plants produce starch to store energy. The starch is present in the form of granules that are insoluble in water at room temperature. Most starches consist of a mixture of two large polysaccharide molecules, amylose and amylopectin. Amylose is an essentially linear molecule consisting of α-D-glucopyranose residues linked together by (1→4) bonds. Amylopectin is the highly branched component of starch. On average, it contains about 4–5% (1→6)α-D linked branch points. It is generally agreed that amylopectin has a cluster type structure (Blanshard, 1987; Manners, 1989), in which short chains are arranged in clusters on longer chains.

Starch granules contain ordered regions, which are semi-crystalline and show birefringence. The overall crystallinity is about 20–45%. Amylose and the residues around the branch points of amylopectin form the amorphous regions in the starch granule. The crystallinity arises mainly from ordered linear segments of amylopectin. These are present in the form of double helices with a length of approximately 5 nm. These double helices are crystallized into thin (~ 5 nm) lamellar domains (Fig. 1), which are visible in transmission electron micrographs (TEM) (Kassenbeck, 1975; Kassenbeck, 1978; Yamaguchi *et al.*, 1979; Oostergetel & van Bruggen, 1989). Results of TEM, optical analysis of electron micrographs and small-angle X-ray scattering (SAXS) suggested that the crystalline lamellae are helically arranged (Oostergetel & van Bruggen, 1987; Oostergetel & van Bruggen, 1989; Oostergetel & van Bruggen, 1993). In this model the amylopectin segments in the crystalline regions are all parallel to the axis of the large helix. The diameter of the helix is ~18 nm, and the spacing between successive turns of the helix is approximately 10 nm. The large helices form a more or less continuous super-helical structure, in which left-handed helices are packed in a tetragonal array (Oostergetel & van Bruggen, 1993).

When heat is applied to a starch suspension, starch undergoes a process known as gelatinization. In a concentrated starch system, the starch granules swell slightly and fill almost the whole sytem, and amylose and amylopectin partly separate (Keetels & van Vliet, 1994). In the course of this process at a fairly well defined temperature, the starch granules lose their birefringence and X-ray pattern, which indicates that the

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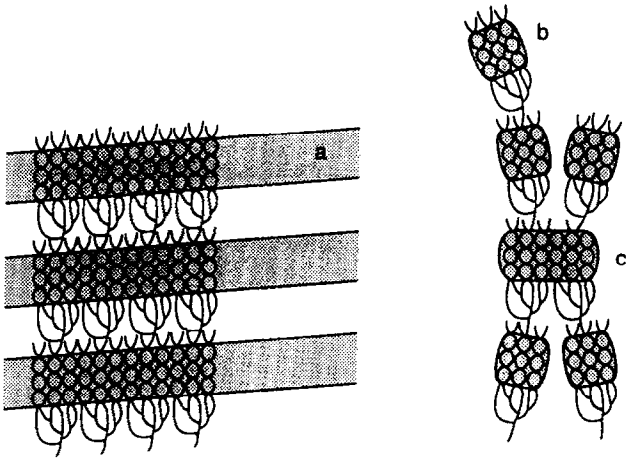

Fig. 1. Highly schematic model for the arrangement of amylopectin in native and retrograded starch. In native starch, the double helices are arranged into thin lamellar domains (a). In retrograded starch, the double helices form small ordered regions being the clusters of linear α-(1→4) glucan chains (b). Probably, adjacent clusters of ordered chains form physical cross-links between amylopectin molecules (c).

ordered regions are disrupted. After complete gelatinization, the periodicity observed by small-angle X-ray scattering is no longer present (Cameron & Donald, 1991), which indicates that also the large helical structures have been disrupted. Thus during gelatinization, the small and long range orders both disappear.

During storage, starch gels regain some of their structural order (retrogradation). The short amylopectin side chains undergo a rapid coil to helix transition (Bulkin *et al.*, 1987; Winter & Kwak, 1987; Goodfellow & Wilson, 1990). WAXS has shown a slow development of crystallinity of the B-form in time, which is closely related to the development of an endothermic transition observed by DSC (Miles *et al.*, 1985; Orford *et al.*, 1987; Roulet *et al.*, 1988). These changes are due to a slow association of the double helices. These reassociation was studied for potato and wheat starch by transmission electron microscopy, DSC and rheology.

MATERIALS AND METHODS

Preparation of concentrated starch gels

To make homogeneous starch gels, dispersions of 3% w/w potato starch (AVEBE, the Netherlands) or 8% w/w wheat starch (Latenstein BV, the Netherlands) in demineralized water were heated to 65°C, while being gently stirred. After cooling to room temperature, sufficient starch was added to obtain suspensions with 30% w/w dry matter. Teflon cylindrical moulds were filled with these suspensions and then heated in an oil bath at 95°C for 90 minutes. After cooling the gels formed were stored at 4, 7 or 20°C.

Large deformation experiments

The Young's modulus E was determined in uniaxial compression tests, performed with a Zwick material testing machine. Test pieces with a height of 20 mm and a diameter of 15 mm were compressed between parallel perspex plates. The initial strain rate was 1.7×10^{-2} s^{-1} and the temperature 20°C. From the force-displacement curve, the compressive stress σ and the Hencky strain ε_h were determined (Luyten *et al.*, 1991) and next the Young's modulus E [$= (d\sigma/d\varepsilon)_{\varepsilon \to 0}$] was calculated. The modulus was determined for 30% potato and wheat starch gels stored at 7°C for 1, 2, 4, 8, 16, 32 or 65 days.

DSC

DSC was performed in a TA Instrument DSC-2910. Just before measurement, approximately 20 mg starch gel was weighed in an aluminium-coated low pressure cup (25 μl). The gels were heated from 20 to 120°C at a scanning rate of 5 K/min. Immediately after heating the gels were rapidly cooled to 5°C. The experiments were performed in two- or threefold after approximately the same storage times as in the compression tests.

Transmission electron microscope

Transmission electron microscopy experiments were performed on 30% w/w potato starch gels that had been stored at 4 or 20°C for 13 days. Small fragments were prepared by first rubbing starch gels into small pieces with a mortar and a pestle. After this treatment, the swollen granules were still intact. They were fragmented by wet mashing in a Potter–Elvehjem tissue homogeniser. Specimens of these fragments for transmission electron microscopy were prepared by negative staining with uranyl acetate as described previously (Oostergetel & van Bruggen, 1989). For ultramicrotomy small (~ 0.1 mm^3) cubes of a starch gel were infused overnight with 2.3 M sucrose, mounted on a copper stub and frozen in liquid nitrogen. Thin (~ 100 nm) frozen sections were cut with a Diatom cryo knife in a Reichert FC 4D/Ultracut E cryo-ultramicrotome at −110°C. Sections were thawed, washed in water, stained with 1% uranyl acetate and air dried. Electron micrographs were recorded at 25,000 or 30,000 × magnification and 80 kV in a Jeol 1200-EX transmission electron microscope.

RESULTS AND DISCUSSION

In transmission electron micrographs (Fig. 2) of small negatively stained fragments of a retrograded 30% w/w potato starch gel, worm-like particles with a diameter of approximately 6 nm and varying lengths are visible. Occasionally, a subdivision into small globular domains can be seen. These worm-like particles are presumably

Fig. 2. Electron micrograph (negative staining) of small fragments of a 30% w/w potato starch gel stored at 4°C for 13 days. Similar micrographs were obtained for a gel stored at 20°C. Bar 200 nm.

the individual amylopectin molecules, the domains being the clusters of linear α-(1→4) glucan chains (Fig. 1). Similar images were obtained from ultra-thin cryo-sections of a starch gel (Fig. 3). In the images presented there is no indication for the presence of helically arranged lamellar domains as seen in electron micrographs of native starch granule fragments and sections (Oostergetel & van Bruggen, 1987). Apparently, the crystallization of amylopectin during retrogradation is limited to individual clusters of linear glucan chains along the amylopectin molecules. The resulting structure is not regular enough to give a discrete reflection in SAXS experiments, consistent with the results of Cameron and Donald (1991). These results thus show that the long range ordering is not regained during starch retrogradation.

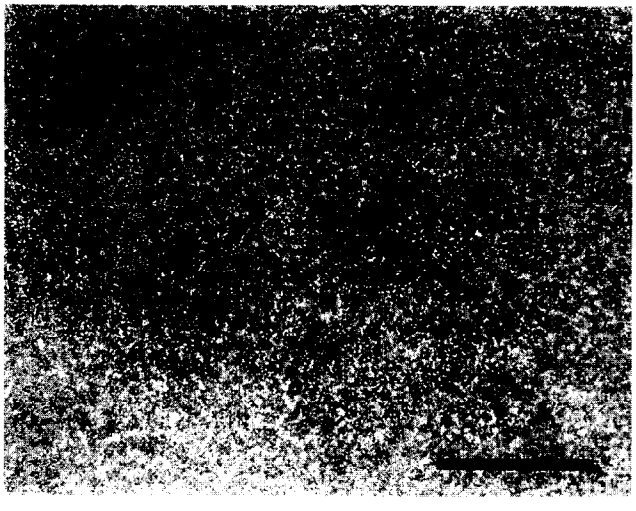

Fig. 3. Electron micrograph of a thin cryo section of a 30% w/w potato starch gel stored at 4°C for 13 days. Cryo sections were thawed and stained with 1% uranyl acetate. Bar 200 nm.

Recrystallization of amylopectin in starch gels results in an increase in stiffness of the gels. In Fig. 4 ($E_t - E_0$) is shown as a function of the melting enthalpy change ΔH, where E_t is the modulus at time t and E_0 is the modulus directly after cooling to 7°C. The relation between the increase in stiffness and the recrystallization of amylopectin is not linear, although they are closely related. The results fit with $(E_t - E_0) = 31(\Delta H)^{1.6}$ for 30% w/w wheat starch gels and with $(E_t - E_0) = 32(\Delta H)^{1.5}$ for 30% w/w potato starch gels. Thus, the relation between the increase in stiffness and the recrystallization of amylopectin is approximately the same for potato and wheat starch gels.

There are two possible mechanisms to explain this relation. In the first place, the close relation between ΔH and $(E_t - E_0)$ and the results obtained from TEM (Figs 2 and 3) suggest that formation of clusters of ordered chains along the amylopectin molecules (Fig. 1) results in stiffening of chains between the entanglements, and with this in an increase of stiffness of the gels.

Another possibility is that during storage of concentrated starch gels ordered regions between adjacent clusters of ordered double helices are formed, resulting in (physical) cross-links between amylopectin molecules (Fig. 1). The close relation between ΔH and $(E_t - E_0)$ would then suggest that the increase in crystallinity is roughly proportional to the increase in the number of cross-links in the swollen granules, assuming that the cross-links are rather homogeneously distributed. The formation of cross-links would thus result in further stiffening of granules. Assuming the cross-links to be microcrystalline regions of parallel chains, the average length of such cross-links would affect the melting enthalpy more than the Young modulus, whereas the number of cross-links would affect the Young modulus more than the melting enthalpy.

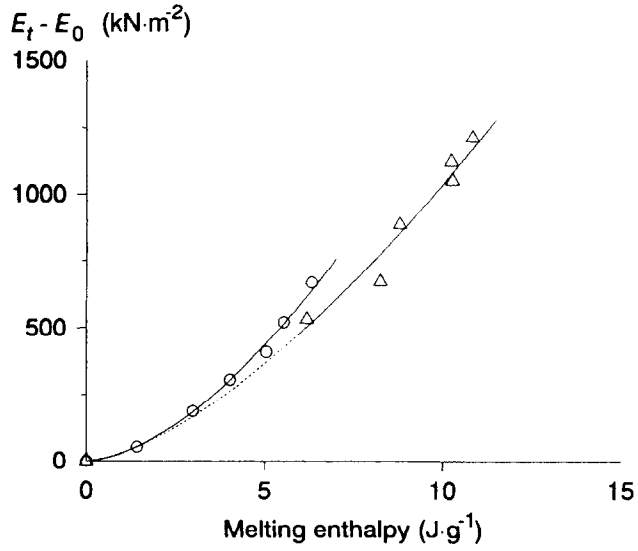

Fig. 4. The increase in stiffness ($E_t - E_0$) plotted as a function of the melting enthalpy change ΔH as determined with DSC for 30% w/w starch gels. (△) potato starch; (○) wheat starch. The storage temperature was 7°C.

The assumption that crystalline regions form cross-links during storage cannot be supported by the transmission electron micrographs, which do not show the formation of ordered regions between adjacent clusters resulting in cross-links. However, the formation of cross-links between two adjacent molecules cannot be excluded.

CONCLUSIONS

During storage of fully disordered starch gels, the short branches of the amylopectin molecules form double helices that become ordered in (semi)-crystalline clusters. The size of these crystalline domains is smaller than those in native starch, and probably mostly limited to the side branches of one main chain. The super-helical structure present in native starch is not regained during retrogradation.

A close relation between the recrystallization of amylopectin, as measured with DSC, and stiffening of concentrated starch gels was observed. Two different processes may explain the increase in stiffness that accompanies recrystallization. The first one is the formation of crystalline clusters along the glucan chains in the amylopectin molecule, which results in stiffening of strands between entanglements. The second process is the formation of cross-links between adjacent clusters. Both mechanisms may play a role simultaneously, although results of TEM suggest that the first mechanism is more prominent. Further research would be required to establish which of the two mechanisms is the more important one.

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